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(54) **Method for controlling molecular weight of polyhydroxyalkanoate constituted of units containing residue of phenyl-, thiienyl-, or cyclohexyl-structure in side chain of molecule**

(57) A method is provided for controlling the molecular weight of a polyhydroxyalkanoate containing at least one of a unit: $-\text{[OCH}((\text{CH}_2)_m\text{R}_1)\text{CH}_2\text{C}(\text{O})]-$ ($m=1-8$; R_1 is a residue having a ring structure of any of phenyl and thiienyl structure), and a unit: $-\text{[OCH}((\text{CH}_2)_k\text{C}_6\text{H}_{10}\text{R}_2)\text{CH}_2\text{C}(\text{O})]-$ ($k=0-8$; R_2 denotes a substituent on the cyclohexyl group including H, CN, NO₂, halogen atom, CH₃, C₂H₅, C₃H₇, CF₃, C₂F₅ or C₃F₇), wherein a microorganism is cultivated in the

presence of a hydroxyl group-containing compound, which is capable of producing the polyhydroxyalkanoate from $\text{R}_3(\text{CH}_2)_q\text{CH}_2\text{CH}_2\text{COOH}$ ($q=1-8$; R_3 contains a residue having a ring structure of phenyl or thiienyl) or $\text{R}_4\text{C}_6\text{H}_{10}(\text{CH}_2)_r\text{CH}_2\text{CH}_2\text{COOH}$ ($r=0-8$; R_4 denotes a substituent on the cyclohexyl group including H, CN, NO₂, halogen, CH₃, C₂H₅, C₃H₇, CF₃, C₂F₅, or C₃F₇).

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Description**BACKGROUND OF THE INVENTION****5 Field of the Invention**

[0001] The present invention relates to a method for controlling the molecular weight of a polyhydroxyalkanoate (PHA), a kind of polyester. More specifically, the present invention relates to a method for controlling the molecular weight of the PHA employing a microorganism which is capable of producing and accumulating the PHA in the cell.

10 Related Background Art

[0002] Many microorganisms have been reported to produce and accumulate poly-3-hydroxybutyric acid (PHB) or other PHA in the cells ("Seibunkaisei Purasutikku Handobukku (Biodegradable Plastics Handbook)", Biodegradable plastics Research Group, NTS K.K., pp.178-197 (1995)). These polymers are useful as various articles molded by melt processing or other processing similarly as conventional polymers. Moreover, these polymers, which are biodegradable, are completely degraded by a microorganism in the nature, not causing pollution in natural environment advantageously, differently from conventional synthetic high polymers. Furthermore, these diodegradable polymers are highly adaptable to a living body and are promising also for a medical soft material and other uses.

[0003] Such PHAs produced by a microorganism are known to have various compositions and structures depending on the kind of microorganism, culture medium composition, cultivation conditions, and other factors in the production. The control of the composition and structure of PHA has been studied for improvement of the physical properties of PHA.

[0004] The PHAs produced by microorganisms are roughly classified into two groups according to the biosynthesis mechanism. One group of PHAs are short-chain-length PHAs (hereinafter referred to as "scl-PHA(s)") typified by polyhydroxybutyric acid (PHB), polyhydroxyvaleric acid (PHV), and copolymers thereof: the other group of PHAs are medium-chain-length PHAs (hereinafter referred to as "mcl-PHA(s)") having medium-chain-length 3-hydroxyalkanoic acid of about 6-14 carbons as the units.

[0005] The former, scl-PHA, is formed from sugars such as glucose and gluconic acid, or acetyl-CoA which is an in-vivo metabolism product of organic acids such as lactic acid, pyruvic acid, and malic acid as the starting material by enzymatic dimerization and reduction into a polymer.

[0006] The latter, mcl-PHA, is formed enzymatically from an alcanoic acid as the starting material by CoA addition, dehydrogenation, and water addition through the β -oxidation pathway, a fatty acid degradation system, into a polymer.

[0007] As mentioned above, the respective groups of PHAs are synthesized through different biosynthesis pathways by action of different enzymes in vivo according to the results of detailed investigation.

[0008] Of the microorganisms producing the latter, mcl-PHAs, some microorganisms are known to produce PHAs having various functional groups and residues.

[0009] Among them, production of the PHAs having an aromatic ring in the unit is actively investigated in recent years.

[0010] Makromol.Chem., 191, 1957-1965 (1990) and Macromolecules, 24, 5256-5260 (1991) describe that Pseudomonas oleovorans produces a PHA containing 3-hydroxy-5-phenylvaleric acid as the unit from 5-phenylvaleric acid as the substrate.

[0011] Macromolecules, 29, 1762-1766 (1996) describes that Pseudomonas oleovorans produces a PHA containing 3-hydroxy-5-(4'-tolyl)valeric acid as the unit from 5-(4'-tolyl)valeric acid as the substrate.

[0012] Macromolecules, 32, 2889-2895 (1999) describes that Pseudomonas oleovorans produces a PHA containing 3-hydroxy-5-(2',4'-dinitrophenyl)valeric acid and 3-hydroxy-5-(4'-nitrophenyl)valeric acid as the units from 5-(2',4'-dinitrophenyl)valeric acid as the substrate.

[0013] Macromol.Chem.Phys., 195, 1665-1672 (1994) describes that Pseudomonas oleovorans produces a PHA copolymer of 3-hydroxy-5-phenoxyvaleric acid and 3-hydroxy-9-phenoxyundecanoic acid from 11-phenoxyundecanoic acid as the substrate.

[0014] Japanese Patent Publication No. 2989175 discloses homopolymers having a 3-hydroxy-5-(monofluorophenoxy)pentanoate (3H5(MHP)P) unit or a 3-hydroxy-5-(difluorophenoxy)pentanoate (3H5(DHP)P) unit, and copolymers having at least the 3H5(MFP)P unit or 3H5(DFP)P unit synthesized by Psudomonas putida (Pseudomonas Genus); and a process for synthesis of the above polymers. Thereby, a polymer having a mono- or di-fluorine-substituted phenoxy group at side chain ends can be synthesized by assimilation of a substituted long-chain fatty acid, the polymer having stereoregularity and water repellency with retention of the high melting point and high processability.

[0015] Cyano- or nitro-substituted polymers are investigated besides the above fluorine-substituted polymers.

[0016] Can.J.Microbiol., 41, 32-43 (1995), and Polymer International, 39, 205-213 (1996) describe production of PHAs containing 3-hydroxy-p-cyanophenoxyhexanoic acid or 3-hydroxy-p-nitrophenoxyhexanoic acid as the monomer unit from octanoic acid and p-cyanophenoxyhexanoic acid or p-nitrophenoxyhexanoic acid as the substrate by em-

ploying *Pseudomonas oleovorans* ATCC29347 strain and *Pseudomonas putida* KT2442 strain.

[0017] *Macromolecules*, 32, 8315-8318 (1999) and *Polymer Preprints, Japan*, 49(5), 1034 (2000) describe capability of *Pseudomonas putida* 27N01 strain to produce PHA copolymers containing 3-hydroxy-5-thiophenoxyvaleric acid and 3-hydroxy-7-thiophenoxyheptanoic acid from 11-thiophenoxyvaleric acid as the substrate.

5 [0018] For practical application of the PHAs, control of the molecular weight is attempted to broaden the application field thereof.

[0019] U.S. Patent No. 6,156,852 discloses the decrease of the number-average molecular weight in biosynthesis of PHB by employing *Ralstonia eutropha*, *Ralstonia latus*, and *Cormamonas testosteroni* as the producing microorganism strain by addition of a diol such as ethylene glycol, neopentyl glycol, propylene glycol, butanediol, hexanediol, and 10 octanediol; butanetriol, polypropylene glycol, glycerol, hydroquinone, benzene-dimethanol, pentaerithritol, and derivatives thereof; or a sugar alcohol such as sorbitol and mannitol to the culture medium. These items are described in detail as chemical reports in *Biotechnology and Bioengineering*, 62, 106-113 (1999), and *International Journal of Biological Macromolecules*, 25, 43-53 (1999).

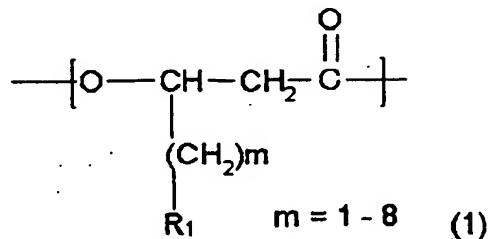
15 [0020] These techniques have merits of controlling the molecular weight in the PHA biosynthesis process without using a chemical substance such as an acid or a base. The PHAs having a functional group such as the phenyl group described above are also required to be controlled in the molecular weight for broadening the practical application field. However, no technique therefore has been developed yet.

SUMMARY OF THE INVENTION

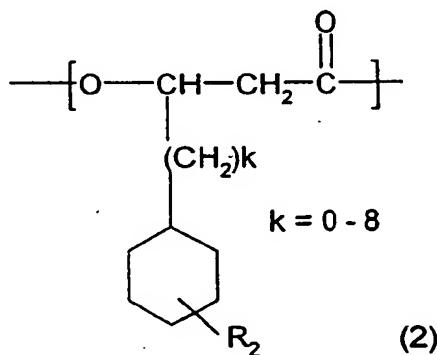
20 [0021] The present invention provides a method for controlling the molecular weight of a polyhydroxyalkanoate having units of a residue containing a phenyl-, thienyl-, or cyclohexyl-structure in the side chain of the molecule.

[0022] After comprehensive study to solve the above problems, the inventors of the present invention achieved the invention described below.

25 [0023] The present invention provides a method for controlling the molecular weight of a polyhydroxyalkanoate containing at least one of 3-hydroxy- ω -substituted alkanoic acid units represented by Chemical Formula (1):



40 (in the above formula, m is an integer selected from the numerical range shown with the Chemical Formula; R_1 is a residue having a ring structure of any one selected from the group consisting of a phenyl structure and a thienyl structure; and in the presence of plural units, m and R_1 are selected independently for the respective units), and 3-hydroxy- ω -cyclohexylalkanoic acid units represented by Chemical Formula (2):



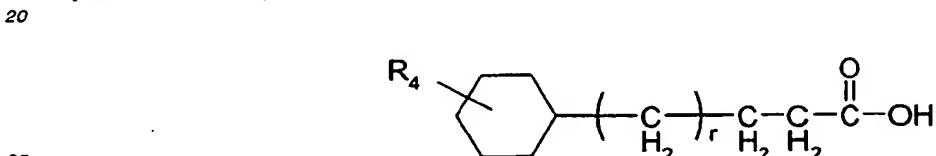
(in the above formula, R_2 denotes a substituent on the cyclohexyl group selected from the group consisting of H atom,

5 CN, NO₂, halogen atom, CH₃, C₂H₅, C₃H₇, CF₃, C₂F₅ and C₃F₇; k is an integer selected from the numerical range shown with the Chemical Formula; and in the presence of plural units, k and R₂ are selected independently for the respective units), wherein a microorganism is cultivated, in the presence of a hydroxyl group-containing compound, which is capable of producing the polyhydroxyalkanoate containing at least one of the units represented by Chemical Formula (1) or (2) from an ω -substituted alkanoic acid represented by Chemical Formula (3):



q = 1 - 8 (3)

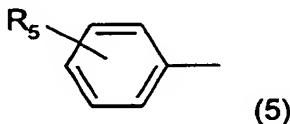
15 (in the above formula, q is an integer selected from the numerical range shown with the Chemical Formula; R₃ is a residue having a ring structure of any one selected from the group consisting of a phenyl structure and a thienyl structure; and in the presence of plural units, q and R₃ are selected independently for the respective units), or ω -cyclohexylalkanoic acid represented by Chemical Formula (4):



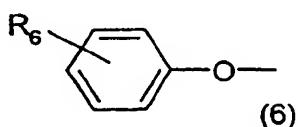
25 r = 0 - 8 (4)

30 (in the above formula, R₄ denotes a substituent on the cyclohexyl group selected from the group consisting of H atom, CN, NO₂, halogen atom, CH₃, C₂H₅, C₃H₇, CF₃, C₂F₅ and C₃F₇; and r is an integer selected from the numerical range shown with the Chemical Formula; and in the presence of plural units, R₄ and r are selected independently for the respective units).

35 [0024] Here, R₁, and R₃ in Chemical Formula (1) or (3), namely the residue having the phenyl structure or the thienyl structure includes specifically the groups represented by Chemical Formulas (5) to (15):
a substituted or unsubstituted phenyl group represented by General Formula (5):



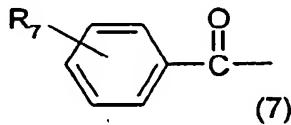
45 (in the above formula, R₅ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, CH₃, C₂H₅, C₃H₇, vinyl, COOR₅₁ (for R₁ only; R₅₁ is a substituent selected from the group consisting of H atom, Na atom and K atom), CF₃, C₂F₅ and C₃F₇; and in the presence of plural units, the above definition is applied independently of the respective units); a substituted or unsubstituted phenoxy group represented by General Formula (6):



55 (in the above formula, R₆ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, CH₃, C₂H₅, C₃H₇, SCH₃, CF₃, C₂F₅ and C₃F₇; and in the presence of plural units, the above

definition is applied independently of the respective units);
 a substituted or unsubstituted benzoyl group represented by General Formula (7):

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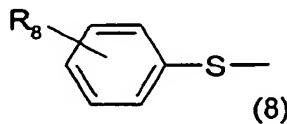
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(in the above formula, R₇ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, CH₃, C₂H₅, C₃H₇, CF₃, C₂F₅ and C₃F₇; and in the presence of plural units, the above definition is applied independently of the respective units);

a substituted or unsubstituted phenylsulfanyl group represented by General Formula (8):

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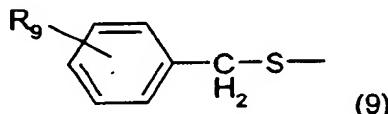


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(in the above formula, R₈ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₈₁, SO₂R₈₂ (R₈₁ is a substituent selected from the group consisting of H, Na, K, CH₃ and C₂H₅; R₈₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units);

a substituted or unsubstituted (phenylmethyl)sulfanyl group represented by General Formula (9):

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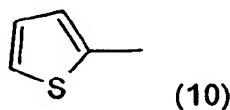
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(in the above formula, R₉ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₉₁, SO₂R₉₂ (R₉₁ is a substituent selected from the group consisting of H, Na, K, CH₃ and C₂H₅; R₉₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units);

a 2-thienyl group represented by Chemical Formula (10):

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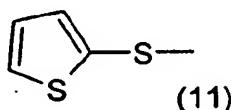
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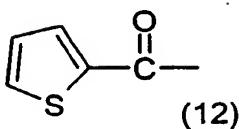
a 2-thienylsulfanyl group represented by Chemical Formula (11):

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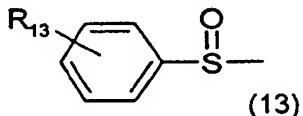
a 2-thienylcarbonyl group represented by Chemical Formula (12):

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a substituted or unsubstituted phenylsulfinyl group represented by General Formula (13) (for R₁ only):

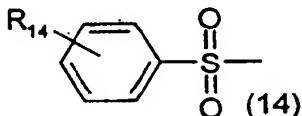
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15 (in the above formula, R₁₃ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₁₃₁, SO₂R₁₃₂ (R₁₃₁ is a substituent selected from the group consisting of H, Na, K, CH₃ and C₂H₅; R₁₃₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units);

20 a substituted or unsubstituted phenylsulfonyl group represented by General Formula (14) (for R₁ only):

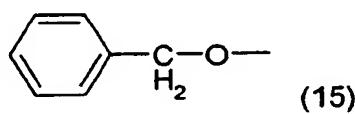
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30 (in the above formula, R₁₄ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, -NO₂, COOR₁₄₁, SO₂R₁₄₂ (R₁₄₁ representing H, Na, K, CH₃ and C₂H₅; R₁₄₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units); and a (phenylmethyl)oxy group represented by Chemical Formula (15):

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[0025] The hydroxyl group-containing compound is at least the one selected from the group consisting of alcohols, diols, triols, alkylene glycols, polyethylene glycols, polyethylene oxides, alkylene glycol monoesters, polyethylene glycol monoesters, and polyethylene oxide monoesters.

[0026] More specifically, the alcohols, diols, and triols may include linear and branched alcohols, diols, and triols of 3-14 carbons.

[0027] The alkylene glycols and alkylene glycol monoesters may be compounds of 2-10 carbons having a linear or branched structure.

[0028] The polyethylene glycols, polyethylene oxides, polyethylene glycol monoesters, and polyethylene oxide monoesters may have a number-average molecular weight ranging from 100 to 20000.

[0029] The hydroxyl group-containing compound is preferably added to the culture medium for cultivation of the microorganism at a concentration preferably from 0.01 to 10%(w/v), more preferably from 0.02 to 5%(w/v). The compound may be added in one batch in the initial stage of the cultivation, or portionwise during the cultivation period.

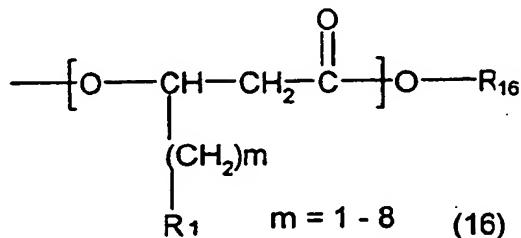
[0030] The microorganism employed in the present invention is the one which has, as described above, capability of producing a polyhydroxyalkanoate containing at least one of 3-hydroxy- ω -substituted alkanoic acid units represented by Chemical Formula (1), and a 3-hydroxy- ω -cyclohexylalkanoic acid unit represented by Chemical Formula (2), from an ω -substituted alkanoic acid, as the source material, represented by Chemical Formula (3), or an ω -cyclohexylalka-

noic acid represented by Chemical Formula (4).

[0031] The polyhydroxyalkanoate of the present invention contains at least one of 3-hydroxy- ω -substituted-alkanoic acid units represented by Chemical Formula (16):

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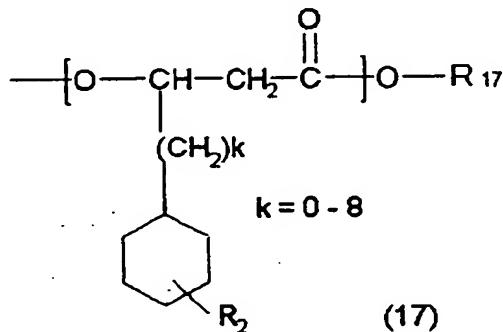
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(in the above formula, m is an integer selected from the numerical range shown with the Chemical Formula; R_1 is defined in above and preferably a residue selected from the group consisting of Chemical formulae (5) to (15); in the presence of plural units, m and R_1 are selected independently for the respective units; and R_{16} is a group derived from a chemical species selected from the group consisting of alcohols, diols, triols, alkylene glycols, polyethylene glycols, polyethylene oxides, alkylene glycol monoesters, polyethylene glycol monoesters, and polyethylene oxide monoesters); and 3-hydroxy- ω -cyclohexylalkanoic acid units represented by General formula (17):

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(in the above formula, R_2 denotes a substituent on the cyclohexyl group selected from the group consisting of H atom, CN, NO_2 , halogen atom, CH_3 , C_2H_5 , C_3H_7 , CF_3 , C_2F_5 and C_3F_7 ; k is an integer selected from the numerical range shown with the Chemical Formula; in the presence of plural units, k and R_2 are selected independently for the respective units; and R_{17} is a group derived from a chemical species selected from the group consisting of alcohols, diols, triols, alkylene glycols, polyethylene glycols, polyethylene oxides, alkylene glycol monoesters, polyethylene glycol monoesters, and polyethylene oxide monoesters).

[0032] The use of the aforementioned microorganism is the essential constitutional requirement of the present invention. Specifically, the microorganisms employed in U.S. Patent No. 6,156,852, Biotechnology and Bioengineering, 62, 106-113 (1999), and International Journal of Biological Macromolecules, 25, 43-53 (1999) do not have capability of producing the polyhydroxyalkanoate containing one or more of the units represented by Chemical Formula (1) or (2) starting from the compound represented by Chemical Formula (3) or (4).

[0033] The microorganisms shown in the above Japanese Patent Publication and the technical literature are reported to produce usually homopolymers and copolymers of poly 3-hydroxybutyric acid (hereinafter referred to as "PHB"), or poly 3-hydroxyvaleric acid (hereinafter referred to as "PHV"). Typically, the biosynthesis pathway for the PHB is summarized as below:

- (1) Acetyl-CoA \rightarrow Acetoacetyl-CoA
- (2) Acetoacetyl-CoA \rightarrow 3-hydroxybutyryl-CoA
- (3) 3-Hydroxybutyryl-CoA \rightarrow poly 3-hydroxybutyric acid.

[0034] On the other hand, the microorganism employed in the present invention biosynthesizes a polyhydroxyal-

kanoate containing one or more of the units represented by Chemical Formula (1) and (2) by taking the compound of Chemical Formula (3) or (4) into the fatty acid degradation pathway called " β -oxidation pathway" by conversion as shown below:

5 <1> Compound (2) → Acyl-CoA
 <2> Acyl-CoA → Enoyl-CoA
 <3> Enoyl-CoA → 3-Hydroxyacyl-CoA
 <4> 3-Hydroxyacyl-CoA

 10 → Polyhydroxyalkanoate of Chemical Formula (1).

[0035] The enzyme participating directly in the above step (3) is a PHB synthase or a short-chain-length PHA synthase, whereas the enzyme employed in the step <4> of the present invention is a PHA synthase or a medium-chain-length PHA synthase. The both enzymes are different from each other in the substrate specificity. This is shown in detail in review papers such as FEMS microbiology Reviews, 103, 217-230 (1992), and Journal of Biotechnology, 65, 127-161 (1998).

[0036] In other words, the microorganism employed in the present invention is completely different from the microorganisms employed in the aforementioned U.S. Patent No. 6,156,852; Biotechnology and Bioengineering, 62 106-113 (1999); and International Journal of Biological Micromolecules, 25, 43-53 (1999) cited above under the heading of 20 Related Background Art in this Patent Specification.

[0037] The microorganism employed in the method of the present invention is not limited, provided that the microorganism has capability of producing the polyhydroxyalkanoate containing at least one of the units represented by Chemical Formula (1) or (2) in the molecule from a source compound represented by Chemical Formula (3) or (4). Among them, particularly preferred are microorganisms of *Pseudomonas* genus, including specifically *Pseudomonas cichorii*, *Pseudomonas putida*, *Pseudomonas fluorescence*, *Pseudomonas oleovorans*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Pseudomonas jessenii*, and so forth. More specifically, particularly preferred are *Pseudomonas cichorii* YN2 (FERM BP-7375), *Pseudomonas cichorii* H45 (FERM BP-7374), *Pseudomonas jessenii* P161 (FERM BP-7376), and *Pseudomonas putida* P91 (FERM BP-7373). These four microorganisms have been deposited to International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology (formerly, National 30 Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology), and are described in Japanese Patent Application Laid-Open No. 2001-371863.

[0038] The microorganism cultivating conditions in the present invention are described below.

[0039] Necessary substrate and nutrients are added as explained below to an inorganic culture medium basically constituted of a phosphate buffer, and an ammonium or nitrate salt.

[0040] As the substrate for production of the intended polyhydroxyalkanoate, the culture medium contains the compound of Chemical Formula (2) preferably at a content ranging from 0.01 to 1%(w/v), more preferably from 0.02 to 0.2%(w/v).

[0041] Usually the culture medium contains preferably the coexisting substrate below as the carbon source and nitrogen source for the microorganism growth, and energy source for the polyalkanoate production at a concentration 40 ranging preferably from 0.1 to 5%(w/v), more preferably from 0.2 to 2%.

[0042] Coexisting Substrate:

- Natural Culture Component: yeast extract, meat extract, malt extract, казаминic acid, casein hydrolyzate, polypeptone, tryptone, peptone, etc.;
- Sugars: aldoses such as glyceraldehyde, erythrose, arabinose, xylose, glucose, galactose, mannose, and fructose;
- Alditol such as glycerol, erythritol and xylitol;
- Aldonic acids such as gluconic acid;
- Uronic acids such as glucuronic acid and galacturonic acid;
- Disaccharides such as maltose, sucrose and lactose;
- Organic acids and salts thereof: pyruvic acid, malic acid, lactic acid, citric acid, succinic acid, oxaloacetic acid, isocitric acid, ketoglutaric acid and fumaric acid, and salts thereof;
- Amino acids: glutamic acid, aspartic acid, and salts thereof, etc.;
- Alkanoic acids: linear or branched alkanoic acids of 4-12 carbons, etc.

[0043] In the present invention, any inorganic culture medium is useful which contains a phosphate salt and a nitrogen source such as an ammonium or nitrate salt. The productivity of PHA can be improved by controlling the concentration of the nitrogen source.

[0044] The cultivating temperature is controlled to be suitable for the growth of the aforementioned microorganism strain, ranging preferably from 15°C to 37°C, more preferably from 20°C to 30°C.

[0045] Any cultivation method may be employed which allows growth of the microorganism to produce PHA, including liquid cultivation and solid cultivation production. The cultivation may be conducted by any kind of cultivation process such as batch cultivation, fed-batch cultivation, semi-continuous cultivation and continuous cultivation. The liquid cultivation method includes a flask-shaking method for oxygen supply, and stirring aeration with a jar fermentor for oxygen supply.

[0046] In another method for producing and accumulating the PHA in the microorganism, the microorganism is allowed to grow sufficiently, then the microorganism mass is transferred to a separate culture medium containing a limited amount of a nitrogen source like ammonium chloride, and the cultivation is continued with addition of the compound for the substrate of the intended units to improve the productivity possibly.

[0047] The objective PHA can be isolated from the microorganism cells after cultivation as described above in a conventional process in the present invention. For example, the most simplest process is extraction with an organic solvent like chloroform, dichloromethane, and acetone. Another organic solvent such as dioxane, tetrahydrofuran and acetonitrile can be useful. In the environment in which use of the organic solvent is not suitable, PHA can be recovered by physically crushing the microorganism cells to remove the cell component other than PHA by any of the methods: treatment with a surfactant like SDS; treatment with an enzyme like lysozyme; by treatment with a chemical such as hypochlorite salts, ammonia and EDTA; and physical crushing of the microorganism cells by ultrasonic crushing, homogenizer crushing, pressure crushing, bead-impact crushing, milling, grinding, and freeze-melting.

[0048] Incidentally, in the present invention, the methods are not limited to the above for cultivation of the microorganism, production and accumulation of PHA in the microorganism cells, and the recovery of PHA by removal of the microorganism cell components other than PHA.

[0049] As shown in the following working examples, the method of the present invention enables control of the molecular weight of polyhydroxyalkanoate which contains a unit having a residue containing a phenyl-, thiethyl-, or cyclohexyl-structure in the side chain of the molecule.

DESCRIPTION OF THE PREFERRED EMBODIMENT

[0050] The composition of the inorganic salt culture medium (M9 culture medium) which was used in a method of the present invention is shown below.

<M9 Culture Medium>

Na ₂ HPO ₄ :	6.3
KH ₂ PO ₄ :	3.0
NH ₄ Cl:	1.0
NaCl:	0.5 g/L, pH=7.0

[0051] For more effective cell growth and PHS production, the minor component solutions should be added to the culture medium in an amount of about 0.3%(v/v) as shown below.

<Minor Component Solution>

Nitrolotriacetic acid:1.5; MgSO₄:3.0; MnSO₄:0.5; NaCl:1.0; FeSO₄:0.1; CaCl₂:0.1; CoCl₂:0.1; ZnSO₄:0.1; CuSO₄:0.1; AlK(SO₄)₂:0.1; H₃BO₃:0.1; Na₂MoO₄:0.1; NiCl₂:0.1 (g/L)

<EXAMPLES>

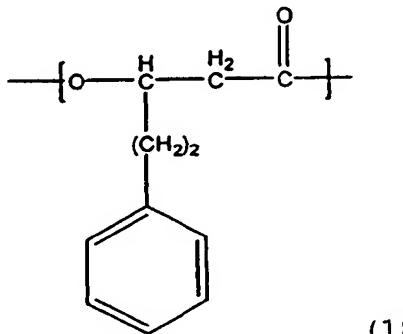
<Example 1>

Molecular Weight Control (1) of Poly 3-hydroxy-5-phenylvaleric Acid (PHPV) by Polyethylene Glycol:

[0052] *Pseudomonas cichorii* YN2 strain was cultivated in an M9 culture medium containing 0.5% of polypeptone at 30°C for 8 hours with shaking. A 1-mL portion of this liquid culture was added to 200-mL portions of an M9 culture medium respectively containing 0.5%(w/v) of polypeptone (Wako Junnyaku K.K.), 0.1%(w/v) of 5-phenylvaleric acid, and 0%, 1%, 2%, or 5%(v/v) of polyethylene glycol 200 (PEG200: average molecular weight 190-210; Kishida Kagaku K.K.) as the molecular weight controlling agent, and cultivated at 30°C for 24 hour in a 500-mL shaking flask. After the cultivation, the microorganism mass was recovered by centrifugation, washed with methanol, and freeze-dried. The dried microorganism mass, after weighing, was stirred in chloroform at 50°C for 24 hours to extract the polymer. The chloroform containing the extracted polymer was filtered and was concentrated by an evaporator. Then cold methanol was added thereto, and solid precipitation formed by addition of the methanol was collected and vacuum-dried to obtain

the intended polymer.

[0053] The obtained polymer was subjected to polymer structure determination by $^1\text{H-NMR}$ (FT-NMR: Bruker DPX400; ^1H resonance frequency: 400 MHz; measured nuclear species: ^1H ; solvent used: CDCl_3 ; reference: capillary-sealed TMS/ CDCl_3 ; measurement temperature: room temperature). Thereby the respective polymers were found to be composed mainly of a homopolymer of 3-hydroxy-5-phenylvaleric acid (hereinafter referred to as "PHPV") (Chemical Formula (18) below):



[0054] The molecular weight of the polymer was measured by gel permeation chromatography (GPC) (Tosoh HLC-8220 GPC, column: Tosoh TSK-gel SuperHM-H, solvent: chloroform, polystyrene basis).

25 [0055] Table 1 shows the weight of the obtained microorganism mass, the weight of the obtained polymer, weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 1

PEG200(%)	CDW(mg/L)	PDW(mg/L)	P/C(%)	Mn	Mw/Mn
0	1238	603	48.7	92000	1.9
1	1022	523	51.1	26000	2.0
2	1007	513	50.9	18000	2.1
5	625	343	54.8	15000	2.1

CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution

<Example 2>

Molecular Weight Control (2) of PHPV by Polyethylene Glycol:

[0056] The experiment was conducted in the same manner as in Example 1 except that PEG600 (average molecular weight: 570-630) was used in place of PEG200 as the molecular weight-controlling agent. According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be composed mainly of a PHPV similarly as in Example 1. Table 2 shows the weight of the obtained microorganism mass, the weight of the obtained polymer, the weight ratio of the polymer to the microorganism mass, the molecular weight and molecular weight distribution of the polymer.

Table 2

PEG600(%)	CDW(mg/L)	PDW(mg/L)	P/C(%)	Mn	Mw/Mn
0	1205	600	49.8	92000	1.9
1	1100	533	48.5	70000	1.9

Table 2 (continued)

PEG600(%)	CDW(mg/L)	PDW(mg/L)	P/C(%)	Mn	Mw/Mn
2	1090	533	48.9	55000	2.1
5	605	321	53.1	49000	2.0

CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution

<Example 3>

Molecular Weight Control (3) of PHPV by Polyethylene Glycol:

[0057] The experiment was conducted in the same manner as in Example 1 except that PEG2000 (average molecular weight: 1800-2200) was used in place of PEG200 as the molecular weight-controlling agent. According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be composed mainly of a PHPV similarly as in Example 1. Table 3 shows the weight of the obtained microorganism mass, the weight of the obtained polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 3

PEG2000(%)	CDW(mg/L)	PDW(mg/L)	P/C(%)	Mn	Mw/Mn
0	1225	602	49.1	92000	1.9
1	1090	519	47.6	80000	2.1
2	1070	522	48.8	67000	2.0
5	618	320	51.8	61000	1.9

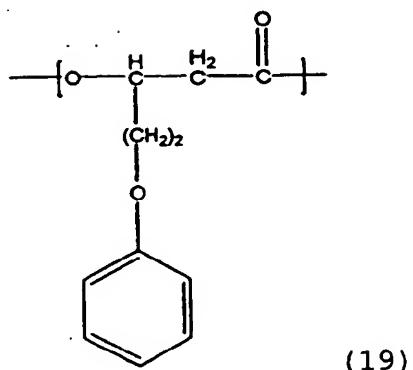
CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution

<Example 4>

Molecular Weight Control of Poly 3-hydroxy-5-phenoxyvaleric Acid by Polyethylene Glycol:

[0058] *Pseudomonas cichorii* YN2 strain, and *Pseudomonas putida* P161 strain were separately cultivated in an M9 culture medium containing 0.5% of polypeptone at 30°C for 8 hours with shaking. A 1-mL portion of one of the liquid cultures was added to a 200-mL portion of an M9 culture medium containing 0.5%(w/v) of polypeptone and 0.1%(w/v) of 5-phenoxyvaleric acid, and containing no PEG200 or containing 1%(v/v) of PEG200 as the molecular weight-controlling agent. Cultivation was conducted at 30°C for 45 hours in a 500-mL shaking flask. After the cultivation, the intended polymer was obtained in the same manner as in Example 1.

[0059] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer of 3-hydroxy-5-phenoxyvaleric acid (Chemical Formula (19) below):



[0060] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0061] Table 4 shows the weight of the obtained microorganism mass, the weight of the obtained polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 4

Micro-organism	PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
YN2	None	760	360	47.4	225000	2.1
	Contained	750	175	23.3	92000	2.0
P161	None	680	150	22.1	160000	1.9
	Contained	530	40	7.5	40000	2.0
CDW: dry weight of the microorganism mass						
PDW: dry weight of the polymer						
P/C: PDW/CDW, Mn: number-average molecular weight						
Mw/Mn: molecular weight distribution						

<Example 5>

Molecular Weight Control of PHPV by Various Molecular Weight-Controlling Agents:

[0062] *Pseudomonas cichorii* YN2 strain was cultivated in an M9 culture medium containing 0.5% of polypeptone at 30°C for 8 hours with shaking. A 1-mL portion of this liquid culture was added to 200-mL portions of an M9 culture medium respectively containing 0.5%(w/v) of polypeptone and 0.1%(w/v) of 5-phenylvaleric acid, and containing no molecular weight-controlling agent or containing 0.1%(v/v) of PEG200 or isopropanol (Kishida Kagaku K.K.) or n-butanol (Kishida Kagaku K.K.) as the molecular weight-controlling agent. Cultivation was conducted at 30°C for 40 hours in a 500-mL shaking flask. After the cultivation, the intended polymers were obtained in the same manner as in Example 1.

[0063] According to ¹H-NMR analysis, the obtained polymers were found to be composed mainly of a homopolymer of PHPV.

[0064] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0065] Table 5 shows the weight of the obtained microorganism mass, the weight of the obtained polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 5

MW controlling agent	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	1170	705	60.3	94000	1.9

Table 5 (continued)

MW controlling agent	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
PEG200	1100	540	49.1	65000	2.1
IPA	1210	600	49.6	79000	1.9
BA	1470	635	43.2	36000	2.3

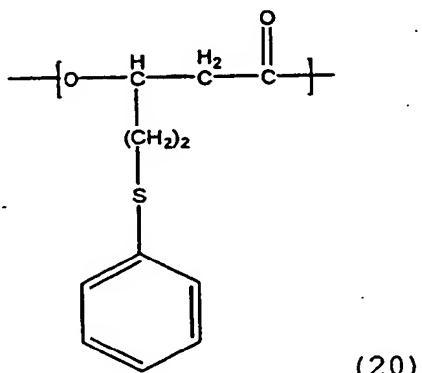
CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution
 IPA: isopropanol, BA: n-butanol

<Example 6>

Molecular Weight Control of Poly 3-hydroxy-5-(phenylsulfanyl)valeric Acid by Various Molecular Weight-Controlling Agents:

[0066] *Pseudomonas cichorii* YN2 strain was cultivated in an M9 culture medium containing 0.5% of polypeptone at 30°C for 8 hours with shaking. A 1-mL portion of this liquid culture was added to 200-mL portions of an M9 culture medium respectively containing 0.5% of polypeptone, 0.1% of 5-(phenylsulfanyl)valeric acid, and containing no molecular weight-controlling agent or containing 0.1%(v/v) of 1,2-butanediol, 1,4-butanediol, 1,6-hexanediol, 1,2,3-butanetriol, ethylene glycol, or ethylene glycol monoethyl ether as the molecular weight-controlling agent. Cultivation was conducted at 30°C for 48 hours in a 500-mL shaking flask. After the cultivation, the intended polymers were obtained in the same manner as in Example 1.

[0067] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer of 3-hydroxy-5-(phenylsulfanyl)valeric acid (Chemical Formula (20) below).



[0068] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
 [0069] Table 6 shows the weight of the obtained microorganism mass, the weight of the obtained polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 6

MW controlling agent	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	1210	590	48.8	150000	2.3
1,2-BD	1200	570	47.5	42000	2.2

Table 6 (continued)

MW controlling agent	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
5	1,3-BD	1215	565	46.5	44000
	1,6-HD	1150	575	50.0	29000
	1,2,3-BT	1090	505	46.3	45000
10	EG	1230	600	48.8	50000
	MEG	1200	590	49.2	53000

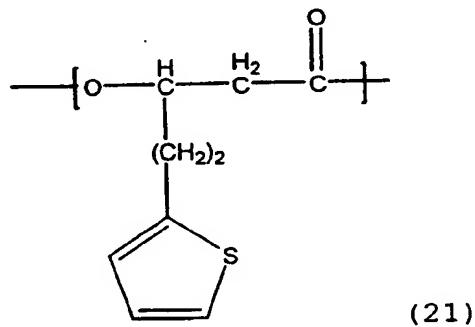
CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution
 1,2-BD: 1,2-butanediol; 1,4-BD: 1,4-butanediol; 1,6-HD: 1,6-hexanediol; 1,2,3-BT: 1,2,3-butanetriol; EG: ethylene glycol; MEG: ethylene glycol monomethyl ether

<Example 7>

Molecular Weight Control of Poly 3-hydroxy-5-(2-thienyl)valeric Acid by PEG:

[0070] *Pseudomonas putida* P91 strain was cultivated in an M9 culture medium containing 0.5% of a yeast extract (Difco) at 30°C for 8 hours with shaking. A 1-mL portion of this liquid culture was added to 200-mL portions of an M9 culture medium containing respectively 0.5% of the yeast extract, 0.1% of 5-(2-thienyl)valeric acid, and containing no molecular weight-controlling agent or containing 0.1%(v/v) of PEG200 as the molecular weight-controlling agent. Cultivation was conducted at 30°C for 45 hours in a 500-mL shaking flask. After the cultivation, the intended polymers were obtained in the same manner as in Example 1.

[0071] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer of 3-hydroxy-5-(2-thienyl)valeric acid (Chemical Formula (21) below).



[0072] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
 [0073] Table 7 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 7

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	600	15	2.5	72000	3.2
Contained	540	16	3.0	30000	2.8

Table 7 (continued)

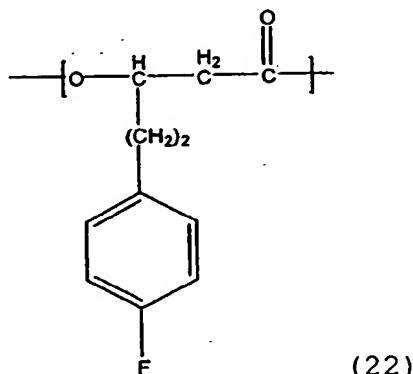
PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
CDW: dry weight of the microorganism mass					
PDW: dry weight of the polymer					
P/C: PDW/CDW, Mn: number-average molecular weight					
Mw/Mn: molecular weight distribution					

<Example 8>

Molecular Weight Control of Poly 3-hydroxy-5-(4-fluorophenyl)valeric Acid by PEG:

[0074] *Pseudomonas cichorii* YN2 strain was cultivated in an M9 culture medium containing 0.5% of polypeptone at 30°C for 8 hours with shaking. A 1-mL portion of this liquid culture was added to 200-mL portions of an M9 culture medium respectively containing 0.5% of D-glucose (Kishida Kagaku K.K.), 0.1% of 5-(4-fluorophenyl)valeric acid, and containing no PEG200 or containing 1%(v/v) of PEG200 as the molecular weight-controlling agent. Cultivation was conducted at 30°C for 48 hours in a 500-mL shaking flask. After the cultivation, the intended polymers were obtained in the same manner as in Example 1.

[0075] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer of 3-hydroxy-5-(4-fluorophenyl) valeric acid (Chemical Formula (22) below).



[0076] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0077] Table 8 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 8

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	790	430	54.4	90000	2.1
Contained	700	390	55.7	22000	2.0
CDW: dry weight of the microorganism mass					
PDW: dry weight of the polymer					
P/C: PDW/CDW, Mn: number-average molecular weight					
Mw/Mn: molecular weight distribution					

<Example 9>

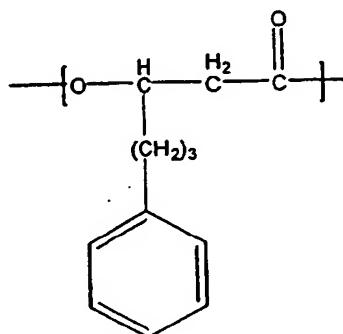
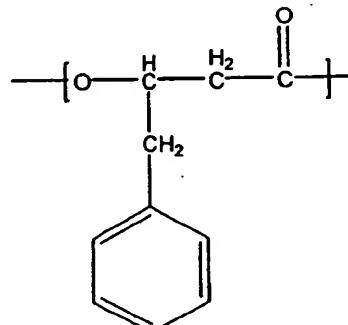
Molecular Weight Control of Poly 3-hydroxy-4-phenylbutyric Acid and Poly 3-hydroxy-6-phenylhexanoic acid by PEG

[0078] The molecular weight-controlling effect of PEG200 was evaluated in the same manner as in Example 8 except that the polymer synthesis substrate was changed to 4-phenylbutyric acid, or 6-phenylhexanoic acid.

[0079] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer

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of 3-hydroxy-4-phenylbutyric acid (Chemical Formula (23) below) or a homopolymer of 3-hydroxy-6-phenylhexanoic acid (Chemical Formula (24) below).



[0080] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0081] Table 9 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 9

Polymer	PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
PHPB	None	420	66	15.7	78000	2.2
	Contained	420	69	16.4	19000	2.1
PHPHx	None	700	72	10.3	80000	2.3
	Contained	660	69	10.5	23000	2.1
PHPB: poly 3-hydroxy-4-phenylbutyric acid PHPHx: poly 3-hydroxy-6-phenylhexanoic acid CDW: dry weight of the microorganism mass PDW: dry weight of the polymer P/C: PDW/CDW, Mn: number-average molecular weight Mw/Mn: molecular weight distribution						

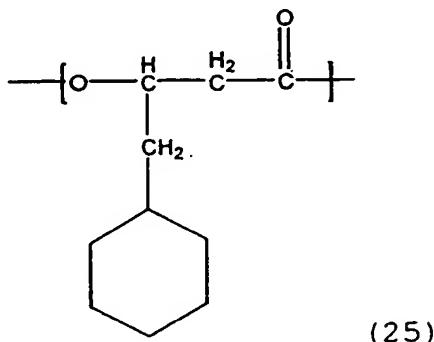
<Example 10>

Molecular Weight Control of Poly 3-hydroxy-4-cyclohexylbutyric Acid by PEG:

55

[0082] The molecular weight-controlling effect of PEG200 was evaluated in the same manner as in Example 8 except that the growth substrate was changed from D-glucose to polypeptone.

[0083] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer of 3-hydroxy-4-cyclohexylbutyric acid (Chemical Formula (25) below).



[0084] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0085] Table 10 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 10

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	820	480	58.5	71000	2.2
Contained	820	430	52.4	18000	2.1

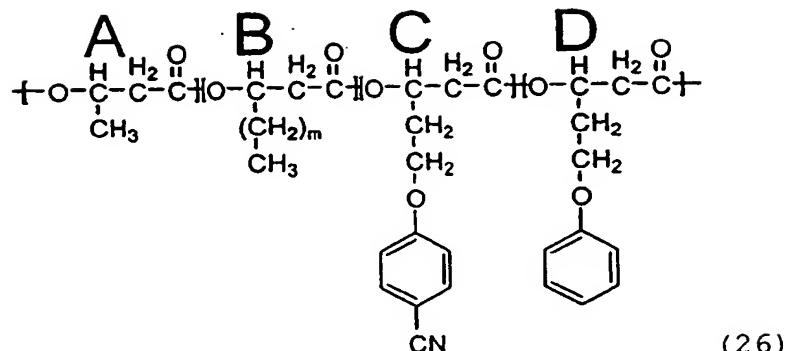
CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution

<Example 11>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-phenoxyvaleric Acid Unit and 3-Hydroxy-5-(4-cyanophenoxy)valeric Acid Unit by PEG:

[0086] *Pseudomonas cichorii* YN2 strain was cultivated in an M9 culture medium containing 0.5% of polypeptone at 30°C for 8 hours with shaking. A 1-mL portion of this liquid culture was added to 200-mL portions of an M9 culture medium containing 0.5% of polypeptone, 0.05% of 5-(4-cyanophenoxy)valeric acid, and 0.05% of 5-phenoxyvaleric acid, and containing no molecular weight-controlling agent or containing 1% (v/v) of PEG200 as the molecular weight-controlling agent. Cultivation was conducted at 30°C for 48 hours in a 500-mL shaking flask. After the cultivation, the intended polymers were obtained by purification in the same manner as in Example 1 and recovery of an acetone-soluble component only.

[0087] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be a PHA containing the units of 3-hydroxy-5-phenoxyvaleric acid and 3-hydroxy-5-(4-cyanophenoxy)valeric acid shown by Chemical Formula (26) below, in which the unit ratio of A:B:C:D = 2:25:5:68 (no PEG-containing medium) and 3:24:7:66 (PEG-containing medium).



[0088] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0089] Table 11 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 11

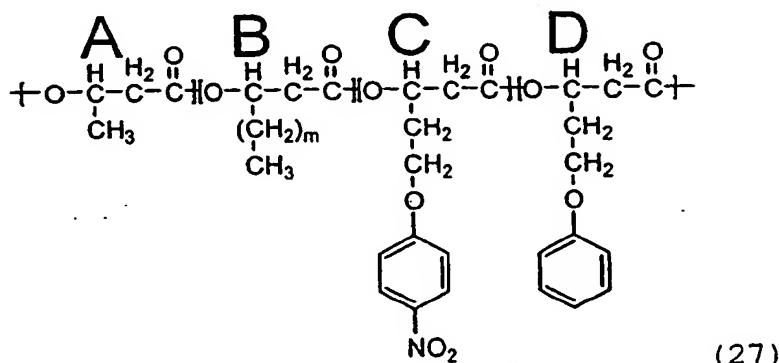
PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	680	110	16.2	72000	2.3
Contained	660	100	15.2	20000	2.1

<Example 12>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-phenoxyvaleric Acid Unit and 3-Hydroxy-5-(4-nitrophenoxy)valeric Acid Unit by PEG:

[0090] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 11 except that 5-(4-cyanophenoxy)valeric acid in the polymer producing substrate was changed to 5-(4-nitrophenoxy)valeric acid.

[0091] According to 1H-NMR analysis, the obtained polymers were found respectively to be a PHA containing the units of 3-hydroxy-5-phenoxyvaleric acid and 3-hydroxy-5-(4-nitrophenoxy)valeric acid shown by Chemical Formula (27) below, in which the unit ratio of A:B:C:D = 2:22:4:72 (no PEG-containing medium) and 4:23:5:68 (PEG-containing medium).



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[0092] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0093] Table 12 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

5 Table 12

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	590	95	16.1	70000	2.2
Contained	570	80	14.0	17000	2.1

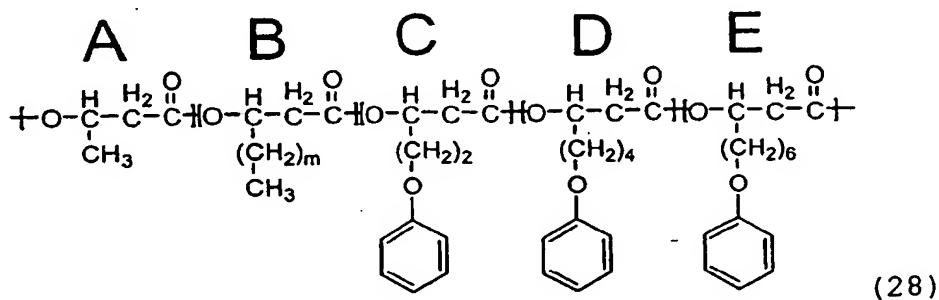
10 CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution

15 <Example 13>

Molecular Weight Control of PHA Containing Units of 3-Hydroxy-5-phenoxyvaleric Acid, 3-Hydroxy-7-phenoxyheptanoic Acid, and 3-Hydroxy-9-phenoxyoctanoic Acid by PEG:

20 [0094] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 10 except that 11-phenoxyundecanoic acid was used as the polymer synthesizing substrate, and *Pseudomonas cichorii* H45 strain was employed as the production strain.

25 [0095] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be a PHA containing the units of 3-hydroxy-5-phenoxyvaleric acid, 3-hydroxy-7-phenoxyheptanoic acid, and 3-hydroxy-9-phenoxyoctanoic acid shown by Chemical Formula (28) below, in which the unit ratio of A:B:C:D:E = 3:1:34:51:11 (no PEG-containing medium) and 3:1:35:52:9 (PEG-containing medium).



40 [0096] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0097] Table 13 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

45 Table 13

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	820	290	35.4	33000	1.9
Contained	815	280	34.4	10000	1.9

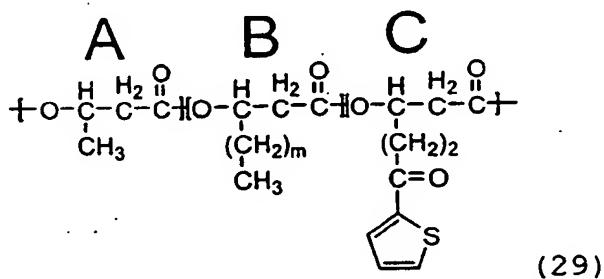
50 CDW: dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution

<Example 14>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-(2-thienoyl)valeric Acid Unit by PEG:

5 [0098] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 8 except that 5-(2-thienoyl)valeric acid was used as the polymer-synthesizing substrate.

[0099] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be a PHA containing a 3-hydroxy-5-(2-thienoyl)valeric acid unit shown by Chemical Formula (29) below, in which the unit ratio of A:B:C = 1:37:62 (no PEG-containing medium) and 1:35:64 (PEG-containing medium).



[0100] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
[0101] Table 14 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 14

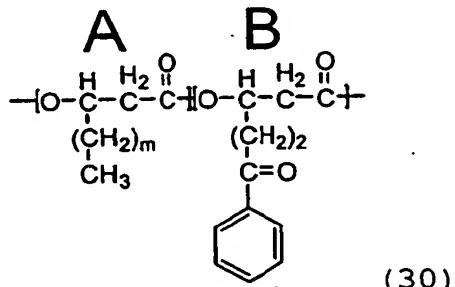
PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	870	95	10.9	110000	2.4
Contained	875	100	11.4	45000	2.2

<Example 15>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-benzoylvaleric Acid Unit by PEG:

[0102] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 8 except that 5-benzoylvaleric acid was used as the polymer-synthesizing substrate.

[0103] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be a PHA containing a 3-hydroxy-5-benzoylvaleric acid unit shown by Chemical Formula (30) below, in which the unit ratio of A:B = 16:84 (no PEG-containing medium) and 15:85 (PEG-containing medium).



[0104] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0105] Table 15 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 15

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	660	170	25.8	330000	3.9
Contained	665	180	27.1	95000	3.7

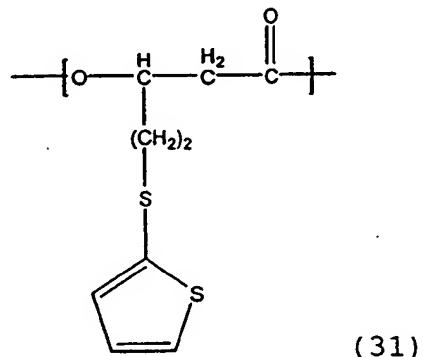
CDW: dry weight of the microorganism mass
PDW: dry weight of the polymer
P/C: PDW/CDW, Mn: number-average molecular weight
Mw/Mn: molecular weight distribution

<Example 16>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-(2-thienylthio)valeric Acid Unit by PEG:

[0106] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 11 except that 5-(2-thienylthio)valeric acid was used as the polymer-synthesizing substrate.

[0107] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be composed mainly of a homopolymer of 3-hydroxy-5-(2-thienylthio)valeric acid shown by Chemical Formula (31) below.



[0108] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0109] Table 16 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 16

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	1100	350	31.8	196000	2.9
Contained	1050	350	33.3	74000	2.6

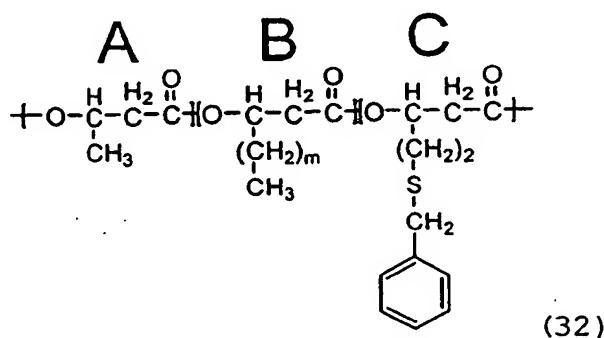
CDW: dry weight of the microorganism mass
PDW: dry weight of the polymer
P/C: PDW/CDW, Mn: number-average molecular weight Mw/Mn: molecular weight distribution

<Example 17>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-[(phenylmethyl)sulfanyl]valeric Acid Unit by PEG:

[0110] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 8 except that 5-[(phenylmethyl)sulfanyl]valeric acid was used as the polymer-synthesizing substrate.

[0111] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be a PHA containing a 3-hydroxy-5-[(phenylmethyl)sulfanyl]valeric acid unit shown by Chemical Formula (32) below, in which the unit ratio of A:B:C = 2:8:90 (no PEG-containing medium) and 2:9:89 (PEG-containing medium).



20 [0112] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
[0113] Table 17 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 17

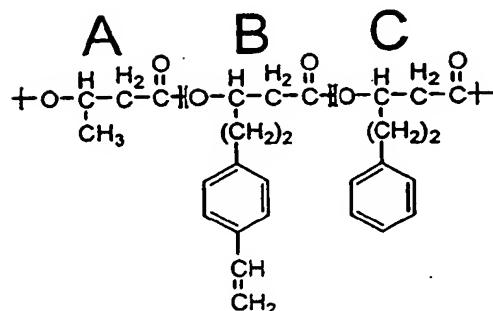
PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	980	440	44.9	15000	3.6
Contained	990	400	40.4	9000	3.2

<Example 18>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-phenylvaleric Acid Unit and 3-Hydroxy-5-(4-vinylphenyl)valeric Acid Unit by PEG

[0114] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 10 except that 5-phenylvaleric acid (0.09%) and 5-(4-vinylphenyl)valeric acid (0.02%) were used as the polymer-synthesizing substrates and the chloroform extraction conditions were changed to 23.5°C and 72 hours.

[0115] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be a PHA containing a 3-hydroxy-5-phenylvaleric acid unit and a 3-hydroxy-5-(vinylphenyl)valeric acid unit as shown by Chemical Formula (33) below, in which the unit ratio of A:B:C = 1:14:85 (no PEG-containing medium) and 1:15:84 (PEG-containing medium).



15 [0116] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
 [0117] Table 18 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 18

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	1200	600	50.0	59000	2.0
Contained	1150	580	50.4	19000	1.9

25 CDW: dry weight of the microorganism mass

PDW: dry weight of the polymer

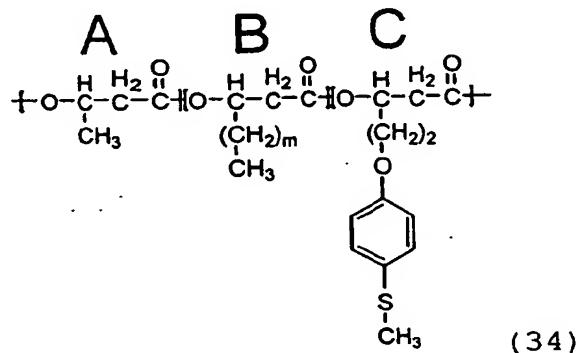
P/C: PDW/CDW, Mn: number-average molecular weight

Mw/Mn: molecular weight distribution

30 <Example 19>

Molecular Weight Control of PHA Containing 3-Hydroxy-5-[(methylsulfanyl)phenoxy]valeric Acid Unit by PEG:

35 [0118] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 10 except that 5-[(methylsulfanyl)phenoxy]valeric acid was used as the polymer-synthesizing substrate.
 [0119] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found respectively to be a PHA containing a 3-hydroxy-5-[(methylsulfanyl)phenoxy]valeric acid unit shown by Chemical Formula (34) below, in which the unit ratio of A:B:C = 8:68:24 (no PEG-containing medium) and 7:66:27 (PEG-containing medium).



55 [0120] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
 [0121] Table 19 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass, and the molecular weight and molecular weight distribution of the polymer.

Table 19

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	990	150	15.2	16000	2.3
Contained	1000	130	13.0	9000	2.1

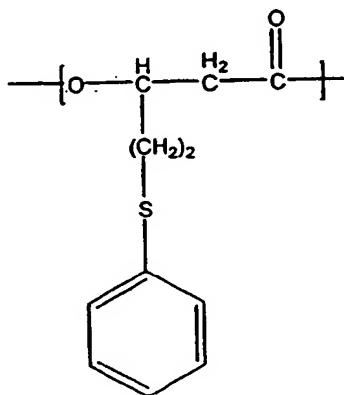
<Example 20>

10 Conversion of Molecular Weight-Controlled Poly 3-hydroxy-5-(phenylsulfanyl)valeric Acid to Poly 3-hydroxy-5-(phenylsulfonyl)valeric Acid:

[0122] The homopolymer of 3-hydroxy-5-(phenylsulfanyl)valeric acid (Chemical Formula (20) below) obtained in Example 6 was converted to poly 3-hydroxy-5-(phenylsulfonyl)valeric acid by oxidation treatment.

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(20)

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[0123] A 400-mg portion of the polyhydroxyalkanoate was dissolved 10 mL of chloroform in a 100-mL eggplant-shape flask. The flask was placed on an ice bath. Thereto was added slowly a solution of 1386 mg of metachloroperbenzoic acid in 20 mL chloroform, and the mixture was stirred gently. After stirring on the ice bath for 75 minutes, were added thereto 100 mL of water and 3020 mg of sodium hydrogensulfite thereto. The mixture was extracted with chloroform to recover the polymer. The polymer was washed two 100-mL portions of ethanol, and vacuum-dried to obtain intended polymer.

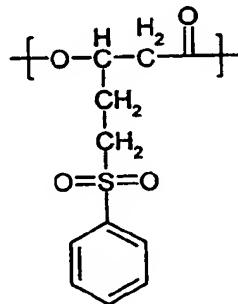
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[0124] The obtained polymers were subjected to polymer structure determination respectively by $^1\text{H-NMR}$ (FT-NMR: Bruker DPX400; resonance frequency: 400 MHz; measured nuclear species: ^1H ; solvent used: CDCl_3 ; reference: capillary-sealed TMS/ CDCl_3 ; measurement temperature: room temperature). Thereby the polymers were found respectively to be a homopolymer of 3-hydroxy-5-(phenylsulfonyl)valeric acid shown by Chemical Formula (35) below:

45

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(35)

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[0125] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.
 [0126] Table 20 shows the weight of the obtained polymer, and the molecular weight and molecular weight distribution of the polymer.

Table 20

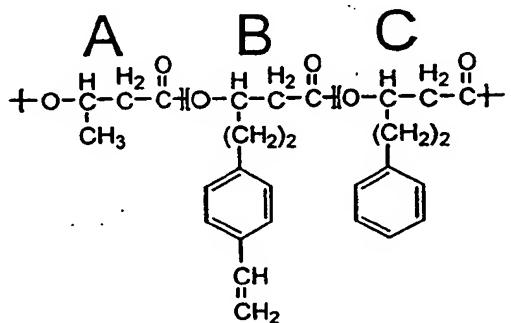
MW controlling agent	Weight (mg)	Mn	Mw/Mn
None	378	135000	2.0
1,2-BD	366	37000	1.9
1,3-BD	389	35000	2.1
1,6-HD	384	26000	1.9
1,2,3-BT	375	42000	2.1
EG	369	48000	2.0
MEG	382	49000	2.1

CDW: Dry weight of the microorganism mass
 PDW: dry weight of the polymer
 P/C: PDW/CDW, Mn: number-average molecular weight
 Mw/Mn: molecular weight distribution
 1,2-BD: 1,2-butanediol; 1,4-BD: 1,4-butanediol; 1,6-HD: 1,6-hexanediol; 1,2,3-BT: 1,2,3-butanetriol; EG: ethylene glycol; MEG: ethylene glycol monomethyl ether

<Example 21>

Conversion of Molecular Weight-Controlled PHA Containing Units of 3-Hydroxy-5-phenylvaleric Acid and 3-Hydroxy-5-(4-vinylphenyl)valeric Acid by Oxidation Treatment to PHA Containing Units of 3-Hydroxy-5-phenylvaleric Acid and 3-Hydroxy-5-(4-carboxyphenyl)valeric Acid:

[0127] The PHA (Chemical Formula 36) obtained in Example 18 containing units of 3-hydroxy-5-phenylvaleric acid and 3-hydroxy-5-(4-vinylphenyl)valeric acid were converted by oxidation treatment to PHA containing units of 3-hydroxy-5-phenylvaleric acid and 3-hydroxy-5-(4-carboxyphenyl)valeric acid.

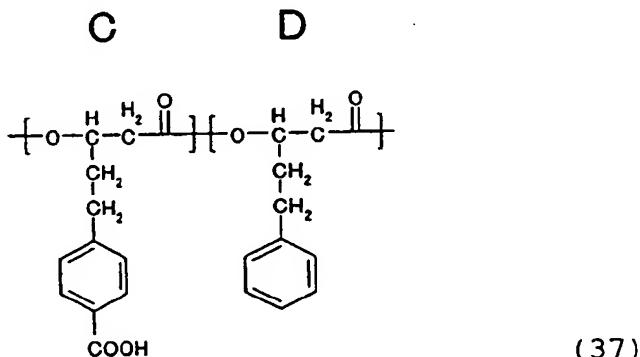


[0128] The oxidative cleavage reaction was conducted as follows. In 100-mL flask, were placed 0.3 g of a polyester containing 3-hydroxy- ω -(4-vinylphenyl)alkanoic acid unit, 0.1923 g of 18-crown-6-ether, and 10.0 mL of dichloromethane, and the mixture was stirred. The flask was placed on an ice bath to keep the reaction system at 0°C. After 30 minutes, 0.1517 g of potassium permanganate was added thereto. The reactor flask was wrapped with an aluminum foil and the reaction mixture was stirred for 21 hours. After completion of the reaction, an aqueous solution of sodium hydrosulfite was added to the reaction mixture, and the reaction mixture was poured into methanol to reprecipitate and recover the polymer. The obtained polymer was purified by dialysis by use of chloroform.

[0129] The structure of the obtained polymer was analyzed by Fourier transform infrared spectroscopy (FT-IR) (Nicolet AV ATAR360 FT-IR). As the result, a new absorption peak of carboxylic acid was observed at 1693 cm⁻¹. This shows the presence of 3-hydroxy- ω -(4-carboxyphenyl)alkanoic acid unit in the obtained PHA.

[0130] The obtained polymer was allowed to react with trisilyldiazomethane, and the reaction product was analyzed by $^1\text{H-NMR}$ (FT-NMR: Bruker DPX400; ^1H resonance frequency: 400 MHz; measured nuclear species: ^1H ; solvent used: CDCl_3 ; reference: capillary-sealed TMS/ CDCl_3 ; measurement temperature: room temperature). Thereby the polymer was found to be a hydroxyalkanoate copolymer containing the unit shown by Chemical Formula (37) below.

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[0131] The reaction product of the obtained polymer with trimethylsilyldiazomethane was evaluated for the average molecular weight by gel permeation chromatography (GPC: Tosoh HLC-8220, column: Tosoh TSK-gel Super HM-H, solvent: chloroform, polystyrene basis).

[0132] Table 21 shows the weight of the obtained polymer, and the molecular weight and molecular weight distribution of the polymer.

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Table 21

PEG200: 1%	Weight (mg)	Mn	Mw/Mn
None	285	32000	1.9
Contained	279	10500	1.7

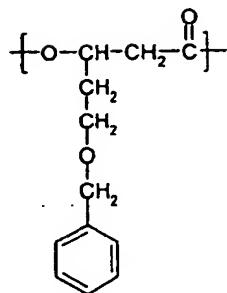
CDW: Dry weight of the microorganism mass
PDW: dry weight of the polymer
P/C: PDW/CDW, Mn: number-average molecular weight
Mw/Mn: molecular weight distribution

<Example 22>

40 Molecular weight Control of PHA Containing 3-Hydroxy-5-[(phenylmethyl)oxy]valeric Acid Unit by PEG:
 45 [0133] The molecular weight-controlling effect of PEG was evaluated by producing a polymer in the same manner as in Example 8 except that 5-[(phenylmethyl)oxy]valeric acid was used as the polymer-synthesizing substrate.
 [0134] According to $^1\text{H-NMR}$ analysis, the obtained polymers were found to be a homopolymer of 3-hydroxy-5-[(phenylmethyl)oxy]valeric acid shown by Chemical Formula (38) below.

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(38)

[0135] The molecular weight of the polymer was measured by GPC in the same manner as in Example 1.

[0136] Table 22 shows the weight of the obtained microorganism mass, the weight of the polymer, the weight ratio of the polymer to the microorganism mass and the molecular weight and molecular weight distribution of the polymer.

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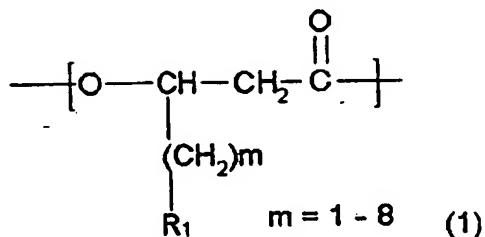
Table 22

25

PEG200: 1%	CDW (mg/L)	PDW (mg/L)	P/C (%)	Mn	Mw/Mn
None	1350	165	12.2	128000	2.4
Contained	1140	125	11.0	52000	2.0

Claims

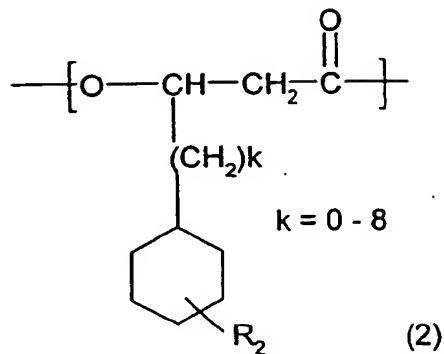
30 1. A method for controlling the molecular weight of a polyhydroxyalkanoate containing at least one of a 3-hydroxy- ω -substituted alcanoic acid unit represented by Chemical Formula (1):



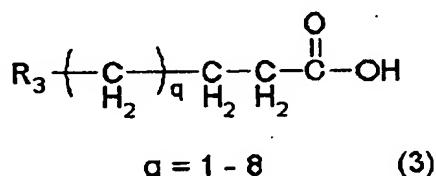
45 (In the above formula, m is an integer selected from the numerical range shown with the Chemical Formula; R1 is a residue having a ring structure of any one selected from the group consisting of a phenyl structure and a thiienyl structure; and in the presence of plural units, m and R1 are selected independently for the respective units), and a 3-hydroxy- ω -cyclohexylalkanoic acid unit represented by Chemical Formula (2):

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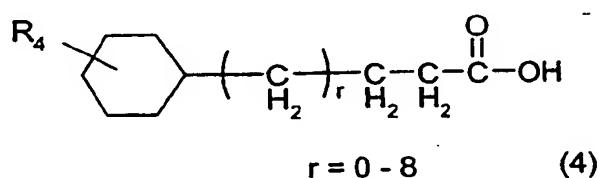
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(in the above formula, R_2 denotes a substituent on the cyclohexyl group selected from the group consisting of H atom, CN, NO_2 , halogen atom, CH_3 , C_2H_5 , C_3H_7 , CF_3 , C_2F_5 and C_3F_7 ; k is an integer selected from the numerical range shown with the Chemical Formula; and in the presence of plural units, k and R_2 are selected independently for the respective units), wherein a microorganism is cultivated, in the presence of a hydroxyl group-containing compound, which is capable of producing the polyhydroxyalkanoate containing at least one of the units represented by Chemical Formula (1) or (2) from an ω -substituted alcanoic acid represented by Chemical Formula (3):



(in the above formula, q is an integer selected from the numerical range shown with the Chemical Formula; R_3 is a residue having a ring structure of any one selected from the group consisting of a phenyl structure and a thienyl structure; and in the presence of plural units, q and R_3 are selected independently for the respective units), or ω -cyclohexylalkanoic acid represented by Chemical Formula (4):

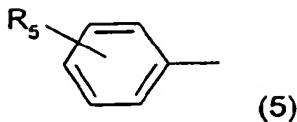


(in the above formula, R_4 denotes a substituent on the cyclohexyl group selected from the group consisting of H atom, CN, NO_2 , halogen atom, CH_3 , C_2H_5 , C_3H_7 , CF_3 , C_2F_5 and C_3F_7 ; and r is an integer selected from the numerical range shown with the Chemical Formula; and in the presence of plural units, R_4 and r are selected independently for the respective units).

50 2. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 1, wherein R_1 and R_3 in Chemical Formula (1) and (3) are residues respectively selected from the group consisting of residues represented by Chemical Formulas (5) to (15):

a substituted or unsubstituted phenyl group represented by General Formula (5):

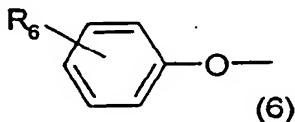
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(in the above formula, R₅ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, CH₃, C₂H₅, C₃H₇, vinyl, COOR₅₁ (for R₁ only; R₅₁ is a substituent selected from the group consisting of H atom, Na atom and K atom), CF₃, C₂F₅ and C₃F₇; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted phenoxy group represented by General Formula (6):

15

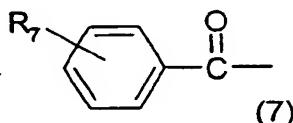


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(in the above formula, R₆ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, CH₃, C₂H₅, C₃H₇, SCH₃, CF₃, C₂F₅ and C₃F₇; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted benzoyl group represented by General Formula (7):

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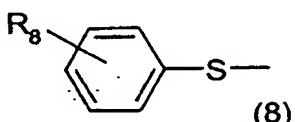
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(in the above formula, R₇ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, CH₃, C₂H₅, C₃H₇, CF₃, C₂F₅ and C₃F₇; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted phenylsulfanyl group represented by General Formula (8):

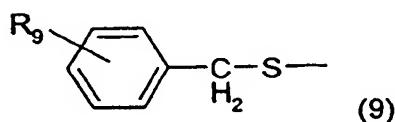
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(in the above formula, R₈ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₈₁, SO₂R₈₂ (R₈₁ is a substituent selected from the group consisting of H, Na, K, CH₃ and C₂H₅; R₈₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted (phenylmethyl)sulfanyl group represented by General Formula (9):

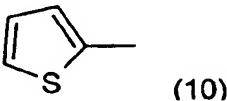


(in the above formula, R₉ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₉₁, SO₂R₉₂ (R₉₁ is a substituent selected from the group consisting of

EP 1 340 776 A1

H, Na, K, CH₃ and C₂H₅; R₉₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units);
a 2-thienyl group represented by Chemical Formula (10):

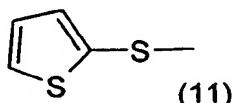
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a 2-thienylsulfanyl group represented by Chemical Formula (11):

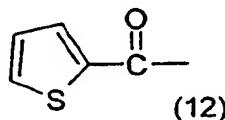
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a 2-thienylcarbonyl group represented by Chemical Formula (12):

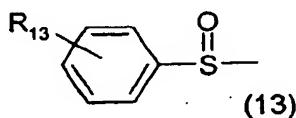
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a substituted or unsubstituted phenylsulfinyl group represented by General Formula (13) (for R₁ only):

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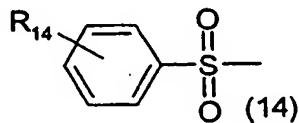
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(in the above formula, R₁₃ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₁₃₁, SO₂R₁₃₂ (R₁₃₁ is a substituent selected from the group consisting of H, Na, K, CH₃ and C₂H₅; R₁₃₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units);

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a substituted or unsubstituted phenylsulfonyl group represented by General Formula (14) (for R₁ only):

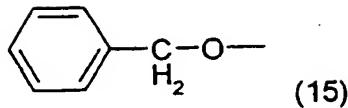
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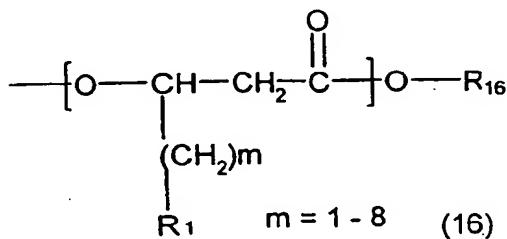
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(in the above formula, R₁₄ denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO₂, COOR₁₄₁, SO₂R₁₄₂ (R₁₄₁ representing H, Na, K, CH₃ and C₂H₅; R₁₄₂ is a substituent selected from the group consisting of OH, ONa, OK, halogen atom, OCH₃ and OC₂H₅), CH₃, C₂H₅, C₃H₇, (CH₃)₂-CH and (CH₃)₃-C; and in the presence of plural units, the above definition is applied independently of the respective units); and

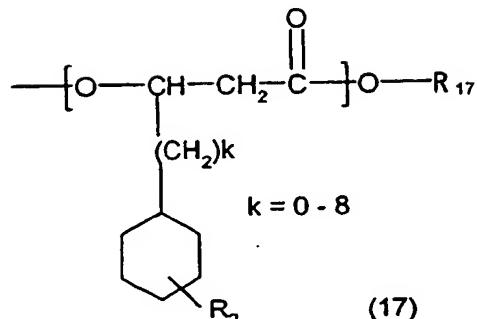
a (phenylmethyl)oxy group represented by Chemical Formula (15):



3. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 1, wherein the hydroxyl group-containing compound is at least the one selected from the group consisting of alcohols, diols, triols, alkylene glycols, polyethylene glycols, polyethylene oxides, alkylene glycol monoesters, polyethylene glycol monoesters, and polyethylene oxide monoesters.
4. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 3, wherein the alcohols, diols, and triols have a linear or branched structure of 3-14 carbons.
- 15 5. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 3, wherein the alkylene glycols and alkylene glycol monoesters have a linear or branched structure of 2-10 carbons, respectively.
- 20 6. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 3, wherein the polyethylene glycols, polyethylene oxides, polyethylene glycol monoesters, and polyethylene oxide monoesters have a number-average molecular weight ranging from 100 to 20000, respectively.
- 25 7. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 1, wherein the hydroxyl group-containing compound is used in the cultivation of the microorganism at a concentration from 0.01 to 10% (w/v), more preferably from 0.02 to 5% (w/v).
- 30 8. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 1, wherein the microorganism belongs to *Pseudomonas* genus.
9. The method for controlling the molecular weight of a polyhydroxyalkanoate according to claim 8, wherein the one or more of *Pseudomonas cichorii* YN2 (FERM BP-7375), *Pseudomonas cichorii* H45 (FREM BP-7374), *Pseudomonas jessenii* P161 (FERM BP-7376), and *Pseudomonas putida* P91 (FREM BP-7373).
- 35 10. A polyhydroxyalkanoate, which contains at least one of a 3-hydroxy- ω -substituted-alkanoic acid unit represented by Chemical Formula (16):



(in the above formula, m is an integer selected from the numerical range shown with the Chemical Formula; R_1 is a residue having a ring structure of any one selected from the group consisting of a phenyl structure and a thieryl structure; in the presence of plural units, m and R_1 are selected independently for the respective units; and R_{16} is a group derived from a chemical species selected from the group consisting of alcohols, diols, triols, alkylene glycols, polyethylene glycols, polyethylene oxides, alkylene glycol monoesters, polyethylene glycol monoesters, and polyethylene oxide monoesters), and a 3-hydroxy- ω -cyclohexylalkanoic acid unit represented by General formula (17):

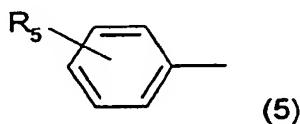


15 (in the above formula, R_2 denotes a substituent on the cyclohexyl group selected from the group consisting of H atom, CN, NO_2 , halogen atom, CH_3 , C_2H_5 , C_3H_7 , CF_3 , C_2F_5 and C_3F_7 ; k is an integer selected from the numerical range shown with the Chemical Formula; in the presence of plural units, k and R_2 are selected independently for the respective units; and R_{17} is a group derived from a chemical species selected from the group consisting of alcohols, diols, triols, alkylene glycols, polyethylene glycols, polyethylene oxides, alkylene glycol monoesters, polyethylene glycol monoesters, and polyethylene oxide monoesters).

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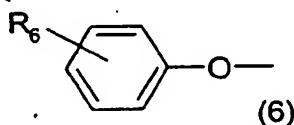
11. The polyhydroxyalkanoate according to claim 10, wherein R_1 in Chemical Formula (16) is a residue selected from the group consisting of residues represented by Chemical Formulas (5) to (15):

25 a substituted or unsubstituted phenyl group represented by General Formula (5):



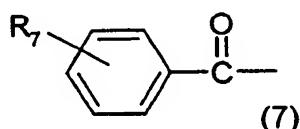
35 (in the above formula, R_5 denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , CH_3 , C_2H_5 , C_3H_7 , vinyl, COOR_{51} (for R_1 only; R_{51} is a substituent selected from the group consisting of H atom, Na atom and K atom), CF_3 , C_2F_5 and C_3F_7 ; and in the presence of plural units, the above definition is applied independently of the respective units);

40 a substituted or unsubstituted phenoxy group represented by General Formula (6):



50 (in the above formula, R_6 denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , CH_3 , C_2H_5 , C_3H_7 , SCH_3 , CF_3 , C_2F_5 and C_3F_7 ; and in the presence of plural units, the above definition is applied independently of the respective units);

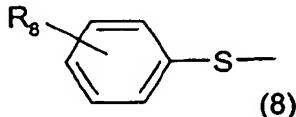
55 a substituted or unsubstituted benzoyl group represented by General Formula (7):



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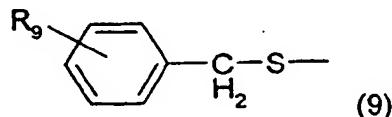
(in the above formula, R_7 denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , CH_3 , C_2H_5 , C_3H_7 , CF_3 , C_2F_5 and C_3F_7 ; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted phenylsulfanyl group represented by General Formula (8):

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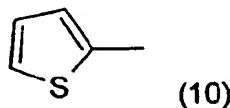
(in the above formula, R_8 denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , $COOR_{81}$, SO_2R_{82} (R_{81} is a substituent selected from the group consisting of H, Na, K, CH_3 and C_2H_5 ; R_{82} is a substituent selected from the group consisting of OH, ONa , OK , halogen atom, OCH_3 and OC_2H_5), CH_3 , C_2H_5 , C_3H_7 , $(CH_3)_2-CH$ and $(CH_3)_3-C$; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted (phenylmethyl)sulfanyl group represented by General Formula (9):

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(in the above formula, R_9 denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , $COOR_{91}$, SO_2R_{92} (R_{91} is a substituent selected from the group consisting of H, Na, K, CH_3 and C_2H_5 ; R_{92} is a substituent selected from the group consisting of OH, ONa , OK , halogen atom, OCH_3 and OC_2H_5), CH_3 , C_2H_5 , C_3H_7 , $(CH_3)_2-CH$ and $(CH_3)_3-C$; and in the presence of plural units, the above definition is applied independently of the respective units);
a 2-thienyl group represented by Chemical Formula (10):

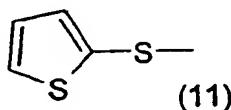
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a 2-thienylsulfanyl group represented by Chemical Formula (11):

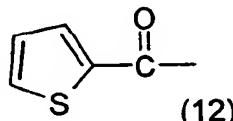
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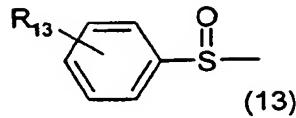
a 2-thienylcarbonyl group represented by Chemical Formula (12):

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a substituted or unsubstituted phenylsulfinyl group represented by General Formula (13) (for R_1 only):

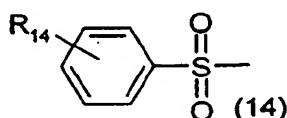
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(in the above formula, R_{13} denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , $COOR_{131}$, SO_2R_{132} (R_{131} is a substituent selected from the group consisting of H, Na, K, CH_3 and C_2H_5 ; R_{132} is a substituent selected from the group consisting of OH, ONa , OK, halogen atom, OCH_3 and OC_2H_5), CH_3 , C_2H_5 , C_3H_7 , $(CH_3)_2-CH$ and $(CH_3)_3-C$; and in the presence of plural units, the above definition is applied independently of the respective units);
a substituted or unsubstituted phenylsulfonyl group represented by General Formula (14) (for R_1 only):

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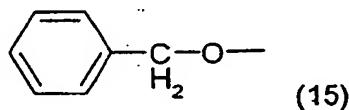


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(in the above formula, R_{14} denotes a substituent on the aromatic ring selected from the group consisting of H atom, halogen atom, CN, NO_2 , $COOR_{141}$, SO_2R_{142} (R_{141} representing H, Na, K, CH_3 and C_2H_5 ; R_{142} is a substituent selected from the group consisting of OH, ONa , OK, halogen atom, OCH_3 and OC_2H_5), CH_3 , C_2H_5 , C_3H_7 , $(CH_3)_2-CH$ and $(CH_3)_3-C$; and in the presence of plural units, the above definition is applied independently of the respective units); and a (phenylmethyl)oxy group represented by Chemical Formula (15):

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EUROPEAN SEARCH REPORT

Application Number

EP 03 00 4348

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	EP 1 113 033 A (CANON KK) 4 July 2001 (2001-07-04) * paragraphs [0060], [0118], [0124] * * paragraphs [0130], [0136], [0141], [0146], [0152], [0158], [0163], [0168], [0173], [0178], [0183], [0188] * * paragraphs [0192], [0231]-[0233]; examples 16-25, 31, 32, 34-37, 41 * * claims 19-23; examples 42-44, 48-50, 55-57, 59, 60, 63, 64, 68 * ---	1-9	C08G63/06 C08G63/682 C08G63/685 C08G63/688 C12P7/62 C08G63/664
X, P	EP 1 275 727 A (CANON KK) 15 January 2003 (2003-01-15) * paragraphs [0047]-[0053]; claims 9, 10, 20, 21; examples 4, 5, 10 *	1-9	
P, X	EP 1 245 605 A (CANON KK) 2 October 2002 (2002-10-02) * paragraphs [0041], [0054]-[0056] * * paragraphs [0102], [0103]; claims 10, 11, 20-22; examples 1-7, 11, 12, 14, 18, 19 *	1-9	
P, X	EP 1 236 755 A (CANON KK) 4 September 2002 (2002-09-04) * paragraphs [0055], [0060], [0079]-[0086]; claims 17, 18, 27, 29, 30; examples 2-5, 10-12, 20-22, 34-39 *	1-9	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C08G C12P C12R C12N
P, X	EP 1 253 161 A (CANON KK) 30 October 2002 (2002-10-30) * paragraphs [0038], [0045], [0080], [0081], [0104], [0105]; claims 9, 10; examples 4, 6, 11, 17, 18, 20 *	1-9 -/-	
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search	Examiner	
MUNICH	24 April 2003	Masson, P	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
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Application Number
EP 03 00 4348

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
P,X	EP 1 253 162 A (CANON KK) 30 October 2002 (2002-10-30) * paragraphs [0056],[0061],[0105],[0106]; claims 9,10; examples 8-10,23 *	1-9	
P,X	EP 1 262 508 A (CANON KK) 4 December 2002 (2002-12-04) * paragraphs [0041],[0042]; claims 13,14,24,25; examples 1-3,13,15-18,23-25 *	1-9	
P,X	EP 1 188 782 A (CANON KK) 20 March 2002 (2002-03-20) * paragraphs [0053],[0056],[0064]-[0066],[0112],[0113]; claims 9,10,12,13; examples 1,2,6,7,16,17 *	1-9	
P,X	EP 1 236 754 A (CANON KK) 4 September 2002 (2002-09-04) * paragraphs [0013]-[0016],[0031]-[0034]; claim 12; example 4 *	1-9	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
P,X	EP 1 236 752 A (CANON KK) 4 September 2002 (2002-09-04) * paragraphs [0026],[0047],[0048]; claims 15,16; example 2 *	1-9	
A	US 5 191 016 A (YALPANI MANSSUR) 2 March 1993 (1993-03-02) * the whole document *	10,11	
	---	-/--	
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search		Examiner
MUNICH	24 April 2003		Masson, P
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	
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EUROPEAN SEARCH REPORT

Application Number
EP 03 00 4348

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A,D	<p>TAKAGI Y ET AL: "BIOSYNTHESIS OF POLYHYDROXYALKANOATE WITH A THIOPHENOXY SIDE GROUP OBTAINED FROM PSEUDOMONAS PUTIDA" MACROMOLECULES, AMERICAN CHEMICAL SOCIETY, EASTON, PA, US, vol. 32, no. 25, 1999, pages 8315-8318, XP001113020 ISSN: 0024-9297 * the whole document * ---</p>	1-11	
X	<p>EP 1 130 043 A (CANON KK) 5 September 2001 (2001-09-05) * paragraphs [0044]-[0045], [0053]-[055.], [0095]-[0099] * claims 15-18; examples 2-4,8-10,14-16 *</p>	1-9	
A	<p>WO 97 07153 A (UNIV MASSACHUSETTS MEDICAL ;GROSS RICHARD A (US); SHI FENGYING (US) 27 February 1997 (1997-02-27) * page 3, line 18 - page 4, line 21 * * page 5, line 28 - page 6, line 14 * * page 7, line 4 - line 18 * * page 9, line 2 - line 13 * * page 10, line 10 - line 38; claims; examples; tables I,IV *</p>	1-11	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	<p>US 5 811 272 A (SIM SANG JUN ET AL) 22 September 1998 (1998-09-22) * the whole document *</p>	1-11	
		-/--	
<p>The present search report has been drawn up for all claims</p>			
Place of search	Date of completion of the search		Examiner
MUNICH	24 April 2003		Masson, P
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			
<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			



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Application Number
EP 03 00 4348

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	<p>SHI F ET AL: "USE OF POLY(ETHYLENE GLYCOL)S TO REGULATE POLY(3-HYDROXYBUTYRATE) MOLECULAR WEIGHT DURING ALCALIGENES EUTROPHUS CULTIVATIONS" MACROMOLECULES, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 29, no. 24, 18 November 1996 (1996-11-18), pages 7753-7758, XP000631125 ISSN: 0024-9297 * the whole document *</p> <p>---</p>	1-11	
A,D	<p>ASHBY R.D. & AL: "A Tunable Switch to Regulate the Synthesis of Low and High Molecular Weight Microbial Polyesters" BIOTECHNOLOGY AND BIOENGINEERING, vol. 62, no. 1, 5 January 1999 (1999-01-05), pages 106-113, XP002239227 * the whole document *</p> <p>---</p>	1-11	
A,D	<p>MADDEN L.A. & AL: "Chain Termination in polyhydroxyalkanoates synthesis: involvement of exogenous hydroxy-compounds as chain transfer agents" INTERNATIONAL JOURNAL OF BIOLOGICAL MOLECULES, vol. 25, pages 43-53, XP002239228 * the whole document *</p> <p>---</p>	1-11	<p>TECHNICAL FIELDS SEARCHED (Int.Cl.7)</p>
X	<p>EP 1 130 042 A (CANON KK) 5 September 2001 (2001-09-05) * paragraphs [0050]-[0058], [0087]; claims 12-15; examples 1-14 *</p> <p>-----</p>	1-9	
<p>The present search report has been drawn up for all claims</p>			
Place of search	Date of completion of the search		Examiner
MUNICH	24 April 2003		Masson, P
CATEGORY OF CITED DOCUMENTS		<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>	
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			

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24-04-2003

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
EP 1113033	A	04-07-2001	JP	2002080571 A	19-03-2002
			CN	1321695 A	14-11-2001
			EP	1113033 A2	04-07-2001
			US	2002022253 A1	21-02-2002
EP 1275727	A	15-01-2003	EP	1275727 A2	15-01-2003
EP 1245605	A	02-10-2002	EP	1245605 A2	02-10-2002
			JP	2003012786 A	15-01-2003
EP 1236755	A	04-09-2002	EP	1236755 A2	04-09-2002
			US	2003013841 A1	16-01-2003
EP 1253161	A	30-10-2002	EP	1253161 A2	30-10-2002
			JP	2003034717 A	07-02-2003
EP 1253162	A	30-10-2002	EP	1253162 A2	30-10-2002
			JP	2003034716 A	07-02-2003
EP 1262508	A	04-12-2002	JP	2003048969 A	21-02-2003
			JP	2003089720 A	28-03-2003
			EP	1262508 A2	04-12-2002
EP 1188782	A	20-03-2002	JP	2002241476 A	28-08-2002
			JP	2003047494 A	18-02-2003
			EP	1188782 A2	20-03-2002
			US	2002160467 A1	31-10-2002
EP 1236754	A	04-09-2002	JP	2002327050 A	15-11-2002
			EP	1236754 A2	04-09-2002
			US	2002164726 A1	07-11-2002
EP 1236752	A	04-09-2002	JP	2002327048 A	15-11-2002
			EP	1236752 A2	04-09-2002
			US	2003059907 A1	27-03-2003
US 5191016	A	02-03-1993	US	5268422 A	07-12-1993
EP 1130043	A	05-09-2001	EP	1130043 A2	05-09-2001
			JP	2001316462 A	13-11-2001
			US	2002065389 A1	30-05-2002
WO 9707153	A	27-02-1997	WO	9707153 A1	27-02-1997
			AU	3406495 A	12-03-1997
US 5811272	A	22-09-1998	AU	3898697 A	20-02-1998

EP FORM 10059
For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.

EP 03 00 4348

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24-04-2003

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
US 5811272 A	WO	9804713 A1	05-02-1998	
EP 1130042 A 05-09-2001	EP	1130042 A2	05-09-2001	
	JP	2002173521 A	21-06-2002	

EP FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82